



Insecticide-treated rodent baits for sand fly control

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ABSTRACT

Rodent baits containing systemic insecticides are potential tools to interrupt the cycle of transmission of *Leishmania* parasites by killing sand flies that take bloodmeals from rodents as adults. Bio-indicators that can be used in conjunction with rodent-targeted sand fly control methods also have been developed to demonstrate that the insecticide treatments are reaching the targeted life stages of sand flies and to quantify the effect of the insecticide treatments on sand fly populations. This article presents new results from a field study on the use of rodent bait containing a systemic insecticide in Kenya. The objective of this field study was to incorporate the fluorescent dye rhodamine B into rodent baits to determine the level of blood feeding by the sand fly *Phlebotomus duboscqi* on targeted rodents, and to demonstrate the effect of rodent bait containing the systemic insecticide ivermectin on bloodfeeding adult females of *P. duboscqi*. Over 50% of the bloodfed females of *P. duboscqi* collected at sites that were treated with rodent baits containing rhodamine B alone were positive for the presence of rhodamine B while no blood-fed females of *P. duboscqi* collected at the sites treated with rodent baits containing rhodamine B plus ivermectin were positive for the presence of rhodamine B. The results of this field trial constitute proof of concept for the targeted control of an epidemiologically significant portion of the population of the sand fly vector of *Leishmania major*, and demonstrate the potential for the interruption of the transmission of *L. major* using applications of systemic insecticide-treated rodent baits.

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1. Introduction

Phlebotomine sand flies belong to a subfamily of small, hematophagous diptera that are biting pests of man as well as the vectors of several pathogens of man and animals. Of the pathogens transmitted by sand flies, the protozoan parasites in the genus *Leishmania* are the most epidemiologically important. Human infection with *Leishmania* parasites can cause disfiguring cutaneous lesions or life-threatening visceral disease. In the absence of a vaccine or effective control methods, leishmaniasis remains an uncontrolled and emerging disease that disproportionately affects the poor [1].

In the Old World, *Leishmania major* is a causative agent of zoonotic cutaneous leishmaniasis (ZCL) that is circulated among populations of burrow-dwelling rodents by adult female sand flies that feed on these rodents. Infections occur in man when he encroaches on this enzootic cycle. In ZCL foci in sub-Saharan Africa, the main

vector of *L. major* is *Phlebotomus duboscqi*, which is closely associated with the rodent reservoirs of *L. major* [2]. Throughout North Africa, the Middle East, and Southwest Asia, a similar enzootic cycle of *L. major* exists involving burrowing gerbils and the sand fly *Phlebotomus papatasi*. Adults of *P. duboscqi* and *P. papatasi* use the burrows of rodents as diurnal resting sites, and larvae have been recovered from inside rodent burrows [3].

There are no effective control or preventive measures currently available in ZCL foci in part because traditional vector control methods recommended by WHO for sand fly control are not effective in prevention of transmission. Insecticide applications targeting adult sand flies and personal protective measures against sand fly bites such as bed nets, insect repellents, and insecticide-treated clothing do not appear to be effective and are often not available to or practical for at-risk populations in low- and medium-income countries [4,5].

Insecticide-treated rodent baits are a possible way to take advantage of the close ecological relationship between rodent reservoirs and sand fly vectors. Laboratory studies have been conducted previously on rodent bait containing systemic insecticides for control of sand flies and on bio-indicators that can be used to trace insecticide treatments to the targeted life stage of sand flies,

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and these laboratory studies provide proof of concept for these techniques and lay the necessary foundation for designing field trials that would produce verifiable results [6–9]. The objective of this study was conduct a field trial in Kenya where rodent baits containing a systemic insecticide and/or a bio-indicator to demonstrate control of sand flies feeding on rodents.

2. Materials and methods

A small-plot field trial of rodent bait containing a systemic insecticide was conducted in Baringo District, Kenya, a semi-arid area in the Kenyan Rift Valley. The ecology of the study location has been described previously by Britch et al. [10]. Two study areas were selected, representing different land uses in the area. All study areas were dominated by acacia trees. The Kenya Agricultural Research Institute study area (KARI; lat 0.47, long 36.00) was comprised of land used for small-scale farming and for forage by goats, and the Rabai study area (Rabai; lat 0.45, long 35.98) was grassy terrain that was surrounded by fences to exclude livestock.

Two sites (50 m radius) were selected at each study area; the sites were at least 500 m apart from each other. Preliminary sampling of the rodents present at each site was conducted over 5 d using 12 Sherman traps (H.B. Sherman Traps Inc., Tallahassee, FL, USA) per site. Sherman traps were baited with a mixture of corn meal, millet seeds, and peanut butter. Daily capture rates of rodents were higher than 75% at each site, and all captured rodents were identified as potential reservoirs of *L. major* in Kenya (*Arvicanthus* sp., *Mastomys* sp., *Tatera* asp., *Taterillus* sp.). Each site had at least one rodent burrow at the center of the site and at least three others that had been located throughout the study site. Rodent burrows were located beneath vegetation, but foraging activity occurred throughout the entire site as indicated by capturing rodents throughout the sites.

One site at each study area was treated with rodent bait (a mixture of corn meal, millet seeds, and peanut butter) containing 5 g/kg rhodamine B (Sigma, St. Louis, MO) plus 0.02 g/kg ivermectin (Merck & Co., Inc., Whitehouse Station, NJ), and the other site was treated with rodent bait containing 5 g/kg rhodamine B alone. Rodent runs and entrances to rodent burrows were located at each site, and at least five bait boxes were placed on active runs at each site. Sites were baited weekly with a total of 300 g per site for 5 months (March–July 2010). Before boxes were re-baited each week, samples of rodent feces were collected from each site (approximately 2 g of feces were collected around each bait box) and stored frozen for detection of rhodamine B or use in sand fly larval bioassays. Two weeks after bait containing rhodamine B plus ivermectin or rhodamine B alone were placed at the sites, rodents were captured to confirm that they had fed on the baits (through visual detection of pink coloration on their fur from ingesting rhodamine B).

Quality control bioassays were conducted to confirm that rodents were feeding on baits throughout the experiment. Rodent feces that had been collected from each site were examined under fluorescence microscopy for the presence of rhodamine B or were used in sand fly larval bioassays [6]. Feces were fed to larvae of a Kenyan strain of *P. duboscqi* obtained from a colony maintained at LSU AgCenter (Baton Rouge, LA, USA). Feces collected from each site and date were pooled, crushed using a glass mortar and pestle, and fed to 10 second instars (13 ± 2 d-old) in a 26 mL plastic vial with a plaster of Paris base. Four vials containing larvae were prepared for each site and date. Larvae were held in an incubator at 28 °C and 90% relative humidity. The survival of larvae was monitored daily, and the percentage of sand flies that emerged as adults was recorded. Any adults that emerged were examined for the presence of rhodamine B using fluorescence microscopy.

The sand fly population was sampled at each site on three consecutive days in March, April, June, and July 2010 using downdraft light traps with UV lights baited with dry ice. In March 2010, sand fly sampling also was conducted 2 weeks prior to treatment of sites with rodent baits. Two traps were run overnight, and all captured sand flies were killed and stored frozen at -80 °C for up to 6 months. The sand flies then were shipped from Kenya to LSU AgCenter, and the specimens were kept on a cold chain while being sorted and examined for the presence of rhodamine B.

Captured bloodfed female sand flies were separated from non-bloodfed females, and all sand flies were examined for the presence of rhodamine B using fluorescence microscopy. Afterwards, sand flies were cleared and identified. The mean number of females of *P. duboscqi* collected, the mean percentage of females that were bloodfed, and the proportion of bloodfed females that were positive for rhodamine B were determined by site and study area. The data were analyzed statistically using analysis of variance performed with the general linear model procedure, and significantly different means were separated using the Tukey multiple comparison procedure [11].

3. Results

Rodents consumed bait containing rhodamine B plus ivermectin or rhodamine B alone weekly throughout the study (March–July 2010). All bait was removed from the bait boxes at the study sites throughout the study period. All of the 194 rodents captured at all sites after baits containing rhodamine B plus ivermectin or rhodamine B alone were placed were marked pink indicating that they had fed on bait containing rhodamine B, and all rodent feces collected at the sites throughout the study were positive for the presence of rhodamine B when they were examined using fluorescence microscopy (Table 1). Bioassays conducted using field-collected feces and larvae from a laboratory colony of *P. duboscqi* further confirmed that rodents had consumed baits throughout the study at every site. All of the 381 adult sand flies that emerged from vials in which larvae were fed rodent feces from sites treated with baits containing rhodamine B alone were positive for the presence of rhodamine B (Table 1). No adult sand flies emerged from vials in which larvae were fed feces of rodents collected from sites treated with baits containing rhodamine B plus ivermectin, indicating that the rodents had ingested ivermectin throughout the study (Table 1).

A total of 1703 sand flies were captured during the study, of which 400 were *P. duboscqi*, 39 were *Phlebotomus martini*, and 1264 were *Sergentomyia* spp. (including *S. bedfordi*, *S. antennatus*, *S. schwetzi*, *S. africanus*, and *S. clydei*). Pre-treatment sampling of the sand fly populations in March 2010 showed that the mean numbers of *P. duboscqi* collected per night at two different study areas (Rabai 13.5 ± 9.1 and KARI 5.0 ± 2.9) were not significantly different from each other ($F = 0.71$, $df = 1$, $P = 0.5946$). There also was no significant difference between the mean number of females of *P. duboscqi* that were collected at the two sites within each study area that were subsequently treated with rodent baits containing

Table 1
Presence (+) or absence (–) of rhodamine B in specimens.

Specimen	RhB sites	RhB + IVR sites
Field-collected ♂ sand flies	–	–
Field-collected ♀ sand flies (unfed)	–	–
Field-collected ♀ sand flies (bloodfed)	+	–
Field-collected rodent feces	+	+
Sand flies reared on rodent feces in lab	+	n/a ^a

^a Not applicable; all sand flies reared on feces of rodents fed a diet containing rhodamine B plus ivermectin died before adult emergence.

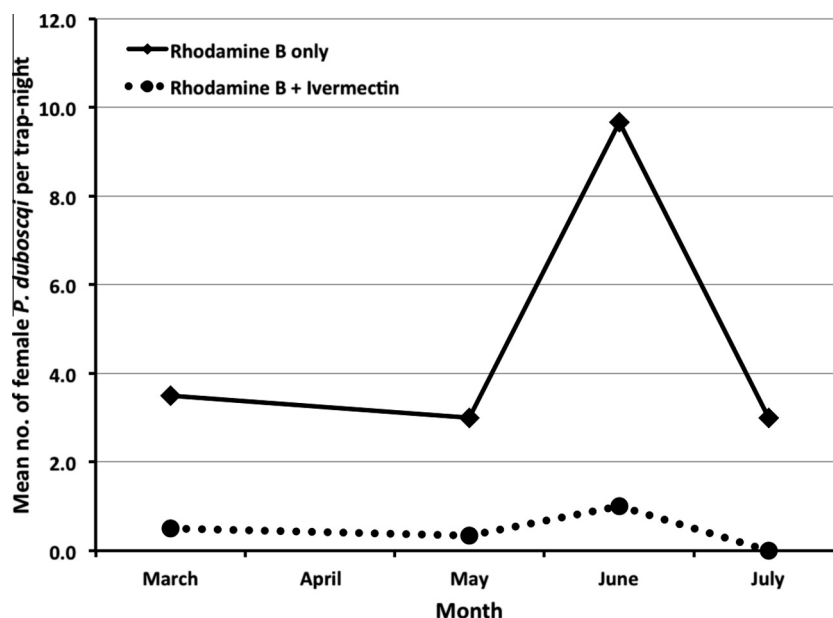


Fig. 1. Mean number of captured females of *P. duboscqi* at the Rabai study location at the site treated with rodent baits containing rhodamine B plus ivermectin or rhodamine B alone.

rhodamine B plus ivermectin or rhodamine B alone. We also did not observe a significant difference between the mean percentages of females of *P. duboscqi* that were bloodfed at the different study areas or at the two sites within each study area ($F = 0.49$, $df = 3$, $P = 0.7055$).

A total of 212 females of *P. duboscqi* were captured during the study at the Rabai site treated with rodent bait containing rhodamine B alone, and 65 were captured at the Rabai site treated with rodent bait containing rhodamine B plus ivermectin (Table 1; Fig. 1). At the KARI sites, a total of 69 females of *P. duboscqi* were captured at the site treated with rodent bait containing rhodamine B alone, and 54 were captured at the site treated with rodent bait containing rhodamine B plus ivermectin (Table 1; Fig. 2). At both Rabai and KARI, there was a peak in the mean number of captured females of *P. duboscqi* in June (Fig. 1; Fig. 2).

An average of $66.3 \pm 28.4\%$ and $50.8 \pm 26.6\%$ of bloodfed females of *P. duboscqi* were positive for the presence of rhodamine B at sites that were treated with rodent baits containing rhodamine B alone at the Rabai and KARI study areas, respectively (Table 1). None of the bloodfed females of *P. duboscqi* that had been collected at any of the sites treated with rodent baits containing rhodamine B plus ivermectin was positive for the presence of rhodamine (Table 2). Additionally, none of the male or unfed female sand flies were found to be positive for the presence of rhodamine B at any of the sites throughout the study.

At the Rabai study area, there was a significant difference between the mean percentage of females of *P. duboscqi* that were bloodfed at the site treated with rhodamine B plus ivermectin ($3.2 \pm 4.8\%$) and the percentage at the site treated with rodent baits containing rhodamine B alone ($22.5 \pm 13.6\%$; $F = 14.4$, $df = 1$,

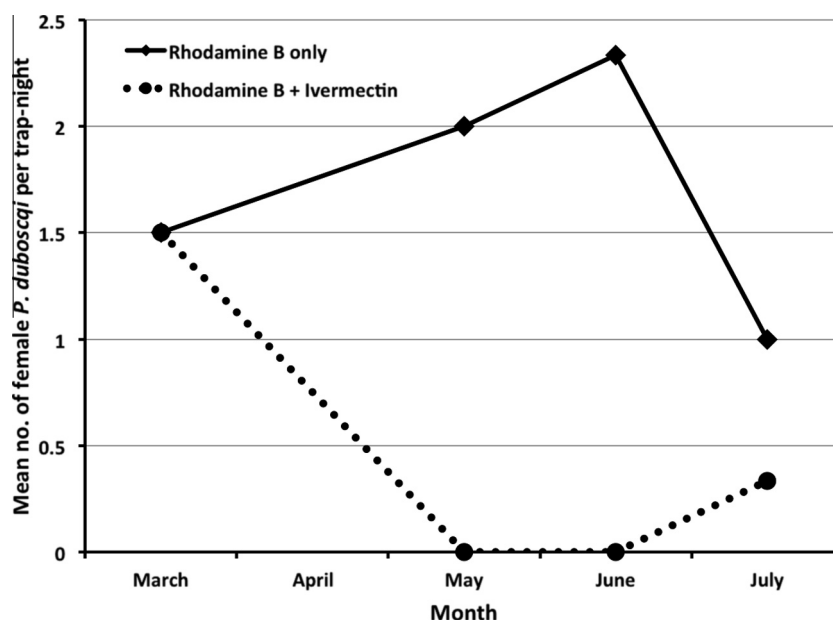


Fig. 2. Mean number of captured females of *P. duboscqi* at the KARI study location at the site treated with rodent baits containing rhodamine B plus ivermectin or rhodamine B alone.

Table 2

Percentage of females of *P. duboscqi* that were bloodfed and the percentage of bloodfed females of *P. duboscqi* that were positive for the presence of rhodamine B at different treatment sites and study areas.

Study area	Total No. females		Percent bloodfed		% of bloodfed sand flies RhB positive		Prevented Fraction ^a
	Rhodamine B alone	Rhodamine B + Ivermectin	Rhodamine B alone	Rhodamine B + Ivermectin	Rhodamine B alone	Rhodamine B + Ivermectin	
Rabai	212	65	22.5 ± 13.6	3.2 ± 4.8	66.3 ± 28.4	0.0 ± 0.0	84.7%
KARI	69	54	29.6 ± 18.0	3.6 ± 8.4	50.8 ± 26.6	0.0 ± 0.0	78.5%

^a Percentage of bloodfed sand flies that were eliminated in the population exposed to rodents targeted with ivermectin-treated baits compared to an untreated population.

$P = 0.0016$; Fig. 3). The odds ratio of captured female sand flies having bloodmeals at the site treated with rodent bait containing rhodamine B plus ivermectin versus the site treated with baits containing rhodamine B alone was 0.153. The preventive fraction (the mean percentage of bloodfed females of *P. duboscqi* that was eliminated as a result of the insecticide-treated rodent baits when compared to the sand fly populations at sites treated with baits containing rhodamine B alone) was 84.7%. The mean number of females of *P. duboscqi* (bloodfed or unfed) collected per trap night at the site treated with rodent baits containing rhodamine B plus ivermectin (8.1 ± 5.1) was lower than the mean number at the site treated with rhodamine B alone, and this difference was nearly statistically significant (21.2 ± 19.3 ; $F = 3.44$, $df = 1$, $P = 0.0823$).

At the KARI study area there was a significant difference between the mean percentage of females of *P. duboscqi* that were bloodfed at the site treated with rhodamine B plus ivermectin ($3.6 \pm 8.4\%$) and rhodamine B alone ($29.6 \pm 18.0\%$; $F = 15.95$, $df = 1$, $P = 0.0009$; Fig. 3). The odds ratio for captured female sand flies having bloodmeals at the different treatment sites was 0.215, and the preventive fraction was 78.5%. There was no significant difference between the mean number of females of *P. duboscqi* collected per trap night at the site treated with rhodamine B plus ivermectin (6.0 ± 4.6) or rhodamine B alone (6.3 ± 3.6 ; $F = 0.02$, $df = 1$, $P = 0.8828$).

4. Discussion

The results of the small-plot trial evaluating rodent bait containing the systemic insecticide ivermectin constitute proof of

concept for the targeted control of an epidemiologically significant portion of the population of the sand fly vector of *L. major*, and the potential for the interruption of the transmission of *L. major* using applications of systemic insecticide-treated rodent baits. After continuous baiting of sites with insecticide-treated baits over a 5 month period, a significant reduction in the percentage of females of *P. duboscqi* that were bloodfed at the two study areas was observed. Furthermore, a trend toward a reduction in the overall population of *P. duboscqi* was observed at one study area.

Three potential scenarios may exist which could explain why we continued to collect bloodfed females of *P. duboscqi* at sites treated with ivermectin. First, it is likely that females of *P. duboscqi* fed on animals other than just the rodents targeted at the study sites. Second, it is possible that we collected female sand flies that had taken a bloodmeal from rodents outside the treatment area, and subsequently infiltrated the study area and were collected by light traps because the treatment area was not large enough. Third, it is possible that a subset of the rodent population (such as neonates) at the study sites were not effectively targeted by the rodent baits, and sand flies took a portion of their bloodmeals from these rodents.

The lack of significant reductions in the overall populations of *P. duboscqi* that was observed at the different study areas appears to be the result of the availability of alternative non-rodent bloodmeal hosts that had not been targeted with insecticide applications. Previous studies have suggested that females of *P. duboscqi* do not exhibit host fidelity, but rather feed on a broad range of hosts [12]. The Rabai study area, where we observed a trend toward a reduced population of *P. duboscqi*, was surrounded by a

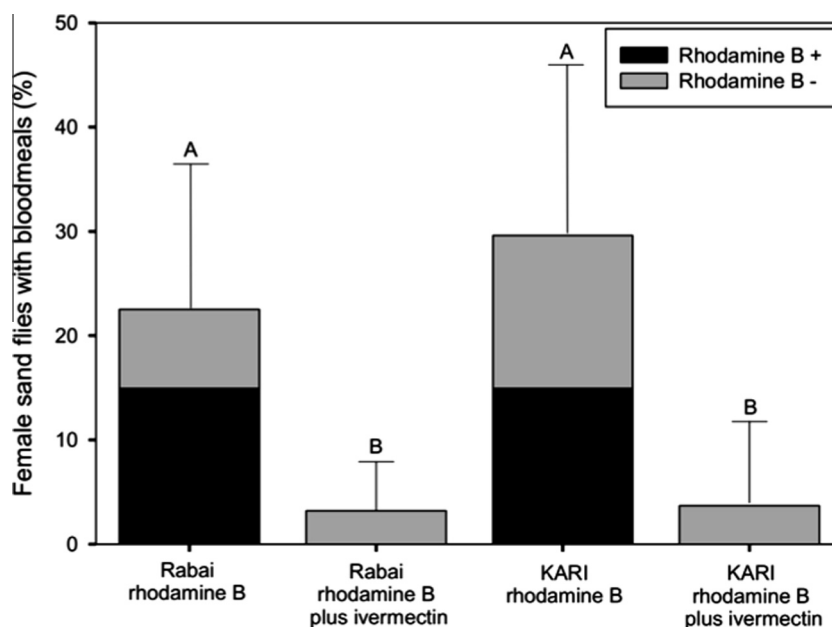


Fig. 3. Mean percentage of captured females of *P. duboscqi* that were bloodfed at sites treated with rodent baits containing rhodamine B plus ivermectin or rhodamine B alone. Values at a study location with the same letter are not significantly different, $P > 0.05$.

fence to exclude livestock, and we did not observe any non-rodent mammals within the study area. The sites at the KARI and Bogoria study areas had large numbers of a variety of non-reservoir animals, including other small mammals, goats, and primates. However, even while it may be unlikely that a population of *P. duboscqi* could be eliminated using systemic insecticide-treated rodent baits, such an intervention could eliminate sand flies that take bloodmeals from baited rodent reservoirs and therefore could have a significant epidemiological impact, breaking the cycle of transmission of *L. major*.

These results also demonstrate the utility of the use of bio-indicators used in conjunction with insecticide-treated rodent baits. By incorporating rhodamine B into baits in this study, it was possible to determine, through the detection of the fluorescent dye in bloodfed female sand flies, the proportion of females of *P. duboscqi* that took bloodmeals from baited rodents at each study area. We also were able to demonstrate that the reduction in the percentage of females of *P. duboscqi* that were bloodfed at sites treated with insecticide-treated rodent baits was similar to the percentage of females of *P. duboscqi* that were positive for the presence of rhodamine B at sites treated with baits containing rhodamine B alone. Furthermore, since none of the sand flies collected at sites treated with rodent baits containing rhodamine B plus ivermectin were positive for the presence of rhodamine B, the use of a bio-indicator allowed us to conclude that there were no instances where the insecticide treatment failed to kill sand flies that took a bloodmeal from a baited rodent.

Our arsenal of available insecticide treatments and bio-indicators has been expanded and improved since the field trial in Kenya in 2010. Fipronil treatment of rodents appears to last up to four times longer than with ivermectin, and the use of fipronil as an active ingredient in rodent bait would allow a monthly baiting scheme as opposed to the weekly baiting scheme that is needed for ivermectin [9]. Furthermore, rubidium permanently marks sand flies that have taken a bloodmeal from a rubidium-treated rodent, compared to rhodamine B, which marks sand flies for approximately 5-d post-bloodmeal. It is possible that the use of rhodamine B as a bio-indicator underestimated the level of blood-feeding by sand flies on baited rodents in the field trial in Kenya, and the use of rubidium in future studies could provide a more accurate measurement. Field trials testing these new chemicals currently are being conducted.

Because of the diversity of rodent-sand fly associations and the complexity of bloodmeal host usages within any individual association, it is unlikely that sand flies could be eliminated using a single control method. Laboratory studies have identified many highly efficacious systemic insecticides that could be incorporated into

rodent bait. Systemic bio-indicators also have been identified and developed as tools to allow the determination of how effective systemic insecticides could be against a specific sand fly population, and as tools to validate observed effects on a sand fly population. Ultimately rodent bait containing a systemic insecticide could be a highly effective approach to target the transmission cycle of *L. major* in many ZCL foci.

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